Evidence for the enhanced biodegradation of ethoprophos and carbofuran in soils from Greece and the UK†

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Abstract: Degradation of carbofuran in a topsoil sample from a previously untreated field site in the UK was characterized by a short lag period followed by rapid degradation. Carbofuran added subsequently to the same soil degraded rapidly without the lag period. In a subsoil sample from the same site, the first application of carbofuran degraded only slowly but degradation rate increased with subsequent treatments and the third dose degraded as rapidly as in the pre-treated topsoil. In similar experiments with ethoprophos, degradation was quite slow and enhanced degradation of subsequent additions of ethoprophos was not observed. A single application of carbofuran in the field in the UK activated soils for rapid biodegradation of the insecticide for at least the subsequent four years. In contrast, in soils from Greece, enhanced degradation was evident six and 18 months after the last carbofuran treatment in the field, but not after three years. Fifty per cent of ethoprophos applied to soils from Greece previously treated with the nematicide was lost within approximately four days, compared with 38 days in a similar, but previously untreated, soil. Very rapid degradation of ethoprophos and carbofuran was observed in soil samples from Greece which had been treated annually with ethoprophos for the last 30 years but with no previous applications of carbofuran. Annual use of the thiocarbamate herbicide EPTC in the same field may have resulted in cross-activation for rapid biodegradation of carbofuran. Very slow degradation of both carbofuran and ethoprophos was observed in soil samples with a history of combined applications of the two pesticides, probably because of their low pH. Fumigation of soil with chloroform, or treatment with the antibacterial antibiotic chloramphenicol, inhibited ethoprophos degradation in a soil where rapid rates of loss had previously been induced, but the antifungal antibiotic cycloheximide had no effect on degradation rate.

Keywords: carbofuran; ethoprophos; pesticides; enhanced biodegradation; stability; soils; subsoils

1 INTRODUCTION

The phenomenon of enhanced biodegradation of pesticides in soil has been well documented for certain carbamate[1,2] and organophosphorus insecticides,[3,4] dicarboximide fungicides,[5] and carbamothioate[6,7] and substituted urea herbicides.[8] In its extreme form, enhanced biodegradation can lead to reduced biological activity against the target plant or organism.[9] Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) is one of the pesticides whose biological efficacy has been reported to be significantly reduced by this phenomenon in many parts of the world.[2,10] In addition to biodegradation in soil, carbofuran may also degrade abiotically, although the published evidence suggests that this occurs only in alkaline soils.[11] In Greece, this insecticide is used in maize (*Zea mays* L) for the control of corn rootworm (*Agriotes* sp), whereas its principal use in the UK is for the control of cabbage root fly (*Phorbia brassicae* Bche) or carrot fly (*Psila rosae* F)[2] in a range of vegetable crops. Carbofuran is a fairly mobile pesticide whose residues have been detected in underground waters in Greece.[12] Its mobility in soils can be explained by weak adsorption and relatively high water solubility which make it available for leaching down the soil profile. Therefore, the ability of subsoil to degrade carbofuran may be important in determining the potential for

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groundwater contamination.

Ethoprophos (*O*-ethyl-*S*,*S*-dipropyl phosphorodithioate) is an organophosphate nematicide used in Greece in potato cultivation mostly for the control of potato cyst nematodes (*Globodera rostochiensis* (Wollenw.) Behrens). It is applied either on its own as a pre-planting granular treatment at doses of 7–10 kg AI ha^{-1}, which is incorporated into the top 7–15 cm of soil, or as a tank mixture with carbofuran at 4 + 4 kg AI ha^{-1}. In contrast to carbofuran, there is only limited evidence for the enhanced biodegradation of ethoprophos in soils.[13]

The main aims of this research were (i) to study the ease of enhancement of carbofuran and ethoprophos degradation in both subsoil and topsoil samples, (ii) to study the stability of the enhanced biodegradation of carbofuran in soils once induced in the field, (iii) to investigate the possible development of enhanced biodegradation of carbofuran and ethoprophos under practical use conditions in Greece, and (iv) to examine the effects of a combined field application of ethoprophos + carbofuran on the development of enhanced biodegradation.

2 EXPERIMENTAL METHODS

2.1 Pesticides, soils and residue analyses

Analytical grade carbofuran (99%, British Greyhound Ltd, Birkenhead) and ethoprophos (99%, Promochem, UK) were used throughout the studies. The soils used were collected from various sites at Horticulture Research International, UK, and from different field locations in Greece. Their properties are shown in Table 1.

Carbofuran residues were extracted from soil (20 g) by shaking with methanol (25 ml) for 50 min on a wrist-action shaker. The samples were left to stand until the soil had settled, after which samples of the clear supernatant were analysed directly by HPLC using a Lichrosorb-RP18 (250 mm × 5 mm) column and acetonitrile + water (60 + 40 by volume) eluant at a flow rate of 1 ml min^{-1}; detection was by UV absorbance at 215 nm. The retention time of carbofuran was 3.90 min. Ethoprophos was extracted from soil in the same way as carbofuran. After this extraction, a sample of the clear methanol extract (5 ml) was shaken vigorously with hexane (5 ml) and distilled water (50 ml) and the hexane extract was then dried over anhydrous sodium sulfate prior to analysis by GLC. This was performed on an AI Cambridge Model 93 chromatograph fitted with a glass column (3 mm × 1.2 m) packed with 3% OV1 on Chromosorb-WHP and with a nitrogen/phosphorus detector. Gas flow rates were: carrier (nitrogen) 50 ml min^{-1}, hydrogen 2 ml min^{-1} and air 450 ml min^{-1}. Temperatures of the injection port, detector and column were 225, 225 and 190°C, respectively. Aliquots (3 µl) were injected manually for each sample and the peak areas compared with those obtained from similar injections of a standard

Soil	*Site*	*pH*	*Organic matter* (%)	*Moisture content* (%)	*Microbial biomass* (*mg C kg^{-1} dry soil*)
A	Deep Slade Subsoil	7.9	2.33	10.99	70.0
B	Deep Slade Topsoil	6.5	2.56	15.43	179.2
C	Long Close control	6.4	3.78	16.71	182.3
D	Long Close treated	5.9	2.45	16.71	105.7
E	Soakwaters control	6.4	3.02	14.51	126.2
F	Soakwaters treated	6.2	2.05	14.51	136.5
G	Sheep Pens control	6.8	2.29	14.79	145.9
H	Sheep Pens treated 1	6.6	1.95	14.79	156.8
I	Sheep Pens treated 2	6.5	2.16	14.79	156.8
J	Little Cherry control	6.6	2.72	15.72	184.7
K	Little Cherry treated 1	6.5	2.52	15.72	139.9
L	Little Cherry treated 2	6.4	2.36	15.72	139.9
M	Thessaloniki Control	8.5	3.57	24.20	–
N	Thessaloniki treated 1	8.3	2.78	22.30	—
O	Thessaloniki treated 2	8.3	3.50	24.80	—
P	Thessaloniki treated 3	8.4	3.59	23.50	—
Q	Thessaloniki treated 4	8.5	3.30	23.50	—
R	Athens treated	8.0	4.96	38.68	—
S	Athens Control	8.2	4.87	32.64	—
T	North Greece 1	5.9	2.35	15.95	—
U	North Greece 2	4.4	2.77	16.83	—
V	North Greece 3	4.4	1.10	12.68	—
W	North Greece Control	5.3	2.06	13.00	—

Table 1. Soil properties

ethoprophos solution (10 mg litre^{-1}); the retention time was 2.3 min.

2.2 Effects of repeated application on degradation of carbofuran and ethoprophos in soil in the laboratory

The soils used were from Deep Slade field at Horticulture Research International (HRI), Wellesbourne (Table 2). Topsoil samples were collected from the 0–20 cm soil layer, and the subsurface samples were collected from the 60–70 cm horizon. There were two topsoil and two subsoil samples which were removed separately using different sampling equipment to avoid cross-contamination. Soil samples were partially air-dried overnight, if necessary, sieved to pass a 3-mm mesh, and their moisture contents and water holding capacities were determined. The partial air-drying was done only if the soils were initially too wet to be sieved at the time of collection. Two replicates (1 kg) of each soil were treated with a solution (4 ml) of carbofuran or ethoprophos in methanol to give a concentration of 15 mg AI kg^{-1} dry soil. This is approximately equivalent to the recommended dose for carbofuran use in the UK (1.5 kg ha^{-1}) incorporated into the top centimetre of soil, and to the highest recommended dose for ethoprophos use in Greece (10 kg ha^{-1}) assuming incorporation into the top 7 cm soil. Soils were left for three to four hours for the solvent to evaporate and then distilled water was added to adjust the moisture content to 40% of the water holding capacity. Soils were mixed by hand initially and then passed three times through a 3-mm-mesh sieve, after which samples (300 g) were transferred to each of three sterile Kilner jars and incubated at 15°C. This provided three sets of two replicates (A, B, C) for each pesticide for each soil. Moisture content was maintained constant throughout the experiment by regular additions of distilled water. The treated soils of group A were sampled periodically over a period of eight weeks and analysed for carbofuran or ethoprophos residues. After 56 days, the remaining samples (groups B and C) were removed from their containers, treated as above with the same dose of carbofuran or ethoprophos, and incubated at 15°C. The soils in group B were sampled periodically for eight weeks, when the soils of group C were treated for the third time with the appropriate pesticide and the degradation rates of these new additions were measured. A modification of the chloroform fumigation-incubation method of Jenkinson and Powlson[14] proposed by Mele and Carter[15] was used for the measurement of the soil microbial biomass at the start of each sequential soil incubation.

2.3 Carbofuran degradation in soils from UK with carbofuran treatment history

Soils were collected from Soakwaters, Little Cherry, Sheep Pens and Long Close fields at HRI, Wellesbourne. All samples came from areas that had received a single dose of carbofuran at some time during the preceding 10 years (Table 2). The treated areas had comprised separate plots of Brussels sprout or broccoli plants for breeding studies, and carbofuran had been applied as a spot application of granules (Yaltox; Bayer, UK; 50 g kg^{-1}) around plants to protect them from damage by cabbage root fly. After harvest and crop clearance, each site had been returned to a standard five-year rotation with cereals before being used again for field experiments. Control soil samples were also collected from areas of the same fields with no history of carbofuran application during the last 10 years. All of these were sampled from areas situated as far away as possible from the previously treated plots, but always within the same soil series. In this way, differences in soil properties such as pH and organic matter content (Table 1) between treated and untreated control soil samples were minimised. The site which had been treated 10 years previously was sampled first and the trowels used for sampling were sprayed with ethanol and dried before and after sampling. Control samples were always collected using separate sterile trowels.

Samples of sieved soil (600 g) from each site received a dose of 15 mg kg^{-1} of carbofuran and were incubated at 15°C with moisture content at 40% of water holding capacity as before. The degradation rate of this new carbofuran addition was determined by periodically removing and analysing subsamples (20 g) for carbofuran residues.

Table 2. Carbofuran treatment history of UK soils

Soil	*Site*	*Pesticide history*
A	Deep Slade subsoil	None known
B	Deep Slade topsoil	No known carbofuran or ethoprophos treatments
C	Long Close control	No carbofuran for the last 10 years
D	Long Close treated	Last carbofuran application 10 years ago
E	Soakwaters control	No carbofuran for the last 10 years
F	Soakwaters treated	Last carbofuran application one year ago
G	Sheep Pens control	No carbofuran for the last 10 years
H	Sheep Pens treated 1	Last carbofuran application eight years ago
I	Sheep Pens treated 2	Last carbofuran application four years ago
J	Little Cherry control	No carbofuran for the last 10 years
K	Little Cherry treated 1	Last carbofuran application six years ago
L	Little Cherry treated 2	Last carbofuran application two years ago

2.4 Carbofuran degradation in soils from Greece with carbofuran treatment history

Soils from Greece with different histories of carbofuran treatment were collected in June 1997 from the farm of the Aristotle University of Thessaloniki. The fields were planted for experimental purposes with maize, and carbofuran had been applied (10G Furadan; FMC, Greece) for the control of corn rootworm (*Agriotes* sp.). Control samples were collected from a nearby field which had no history of carbofuran application. The treatment histories of the soils are shown in Table 3. After sampling, soils were stored moist in the dark at 4°C overnight until they were transported to UK the next day. All soils were handled in an identical way to the UK soils, and received a standard dose of 15 mg kg^{-1} of carbofuran whose degradation was measured as described before.

2.5 Ethoprophos degradation in soil from Greece with previous ethoprophos treatment

The soils used in this study were obtained from an experimental site at the Agricultural University of Athens in April 1997. Details of the pesticide and crop histories of the samples are given in Table 4. One of the samples had received a single application of ethoprophos (720 g litre^{-1} EC, MOCAP, Rhone Poulenc, Greece) added to a potato crop in September 1996, whereas the other had received no ethoprophos applications during the last five years. Two subsamples (300 g) of each soil were treated with a dose of 5 mg kg^{-1} ethoprophos, and incubated at 15°C. The degradation rate of this fresh addition was determined by removing and analysing subsamples (20 g) for ethoprophos residues at intervals over the subsequent 52 days.

2.6 Ethoprophos and carbofuran degradation in Greek soils from potato monocultures

Soil samples were collected in October 1997 from a potato monoculture area in Northern Greece. Soil samples were taken from commercially cultivated fields where ethoprophos (10G Mocap; Rhone Poulenc, Greece) or a combination of ethoprophos + carbofuran (10G Furadan; FMC, Greece) as a tank mixture, was applied annually at planting time for the control of potato cyst nematodes. Control samples were collected from a field within the same area. Details of the pesticide treatment history of each sample are given in Table 5. Duplicate samples (300 g) of each soil were treated either with 10 mg kg^{-1} carbofuran or 5 mg kg^{-1} ethoprophos. All samples were incubated at 15°C with moisture content at 40% of water holding capacity. Degradation rates of the two pesticides were determined as before.

2.7 The effect of antibiotics on degradation of ethoprophos

Further subsamples (4 × 400 g) from soil T (Table 5) were taken and a different treatment was applied to each before a fresh ethoprophos dose was added. The first sample was fumigated under vacuum with chloroform for one week at 30°C in a vacuum desiccator. The soil was contained in a polyester fine mesh net, and the chloroform (2 × 50 ml) was con-

Soil	*Site*	*Carbofuran treatment and cropping history*
M	Thessaloniki control	No application history Continuous cereals
N	Thessaloniki treated 1	Annual application for the last eight years Continuous maize
O	Thessaloniki treated 2	Last application six months ago One year cereals – maize rotation
P	Thessaloniki treated 3	Last application 18 months ago One year cereals – maize rotation
Q	Thessaloniki treated 4	Last application three years ago No crop, cereals

Table 3. Carbofuran treatment and cropping history of soils from Thessaloniki, Greece

Site	*Soil*	*1993–1994*	*1994–1995*	*1995–1996*	*1996–1997*
R	Athens treated	—	—	—	Ethoprophos; Mocap 72% (10 kg ha^{-1})
	Crop	–	Vegetables	Wheat	Potatoes
S	Athens control	–	–	Carbofuran; Curater 10% (20 kg ha^{-1})	Carbofuran; Curater 10% (70 kg ha^{-1})
	Crop	Tomatoes	Wheat	Carrots	Potatoes

Table 4. Pesticide treatment and cropping history of soils from Athens, Greece

Table 5. Pesticide treatment history of soils used for continuous potato cultivation in northern Greece

Soil	*Site*	*Pesticide history*
T	North Greece 1	Annual ethoprophos application for the last 30 years (7 kg AI ha^{-1})
U	North Greece 2	Annual ethoprophos application for the last seven years (7 kg AI ha^{-1})
V	North Greece 3	Combined application of ethoprophos + carbofuran for the last seven years (4 + 4 kg AI ha^{-1})
W	North Greece control	1984–94: No crop 1995–97: Organic potato cultivation

tained in two beakers (100 ml). Other soil samples were treated with aqueous solutions of the antibiotics chloramphenicol (10 ml; 4800 mg $litre^{-1}$) or cycloheximide (10 ml; 4800 mg $litre^{-1}$) to give an antibiotic concentration of 120 mg kg^{-1}. Soils were then mixed and incubated at 15°C for three days. Finally the last sample was left untreated to serve as a control. After all pre-treatments had been completed, ethoprophos was added to the samples (4 ml; 500 mg $litre^{-1}$ solution in methanol as before) to give a concentration of 5 mg kg^{-1}. The soils were then mixed separately by hand, and their moisture content was adjusted to 40% of their water holding capacity by addition of sterile distilled water. All samples were divided into two replicates of 200 g which were transferred to sterile polypropylene bottles and incubated at 15°C. Fumigated soils were handled in a laminar flow bench to maintain sterile conditions as far as possible, and sterile distilled water was used to adjust the moisture contents. At regular intervals, subsamples (20 g) were removed from each polypropylene container and analyzed for ethoprophos residues by GLC as before.

3 RESULTS AND DISCUSSION

3.1 Repeated application of carbofuran and ethoprophos in topsoil and subsoil

The results from the experiment with sequential applications of carbofuran and ethoprophos to soils are shown in Fig 1. With the first application of carbofuran in the topsoil samples, there was an initial short lag phase followed by rapid degradation and residues had disappeared by 25 days. There was no lag phase following the second and third applications of carbofuran to this soil. In the subsoil sample, only 25% of the first dose of carbofuran had degraded after 56 days. However the second dose plus the residual amount from the first addition degraded completely within the next 31 days. Degradation of

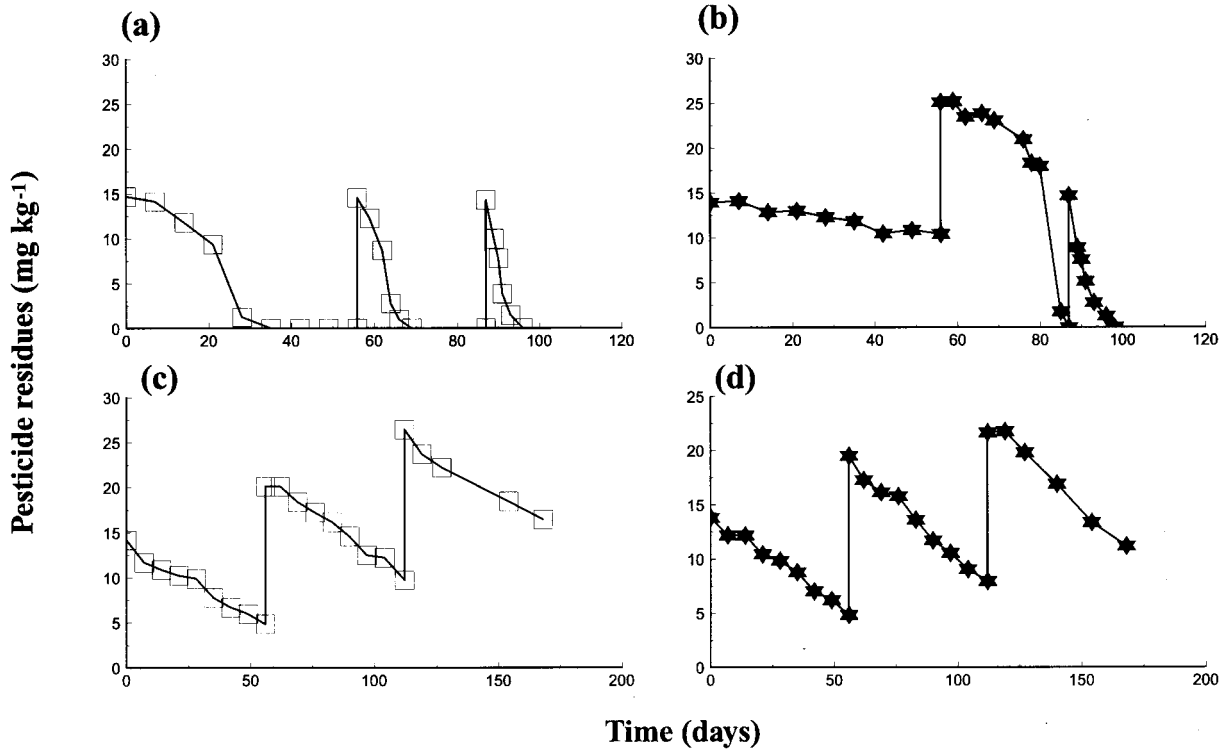

Figure 1. Degradation of (a, b) carbofuran and (c, d) ethoprophos in (□) topsoil and (★) subsoil after three repeated applications in the laboratory.

the third dose was complete in about 10 days, with the pattern of loss very similar to that observed in the topsoil. These results agree with other findings that carbofuran degradation in samples taken from deeper zones in the soil profile is characterized by a much longer lag phase in comparison with surface soil samples (0–10 cm).[16] Locke and Harper[17] have attributed differences in metribuzin degradation in different soil horizons to reductions in microbial activity with increasing soil depth. The pattern of a lag phase in carbofuran degradation following its first introduction to soil suggests that the population of carbofuran-degrading micro-organisms needs time to develop the ability to degrade the insecticide either by enzyme induction, or by proliferation of the active population.[18] Slower development in the subsoil samples suggests that the population effect may be the more important. With ethoprophos, there was no difference in degradation rate between topsoil and subsoil samples. There was also a gradual decrease in degradation rate in both the topsoil and subsoil with each sequential treatment, which resulted in the accumulation of the high ethoprophos levels of 25–30 mg kg^{-1} in the soils.

Measurements of the soil microbial biomass at the beginning and at the end of the experiment are shown in Fig 2. There was a significant decrease in the size of the microbial biomass over this period in the ethoprophos-treated soils. There was also a small reduction in biomass in the carbofuran-treated soils, but this was not statistically significant. Reductions in biomass of 20–30% are often recorded during extended soil incubation studies,[19] and the reductions with ethoprophos in both the topsoil and the subsoil (Fig 2) were greater than this. Carbofuran has been reported to have minimal adverse effects on soil microbial biomass,[20] whereas there is some evidence in the literature for a significant effect of ethoprophos on soil microbial communities.[21] The present results are consistent with these earlier observations. The lack of enhanced degradation of ethoprophos after three repeated applications may have resulted from an inhibitory effect of the nematicide on the soil microflora under the present experimental conditions.

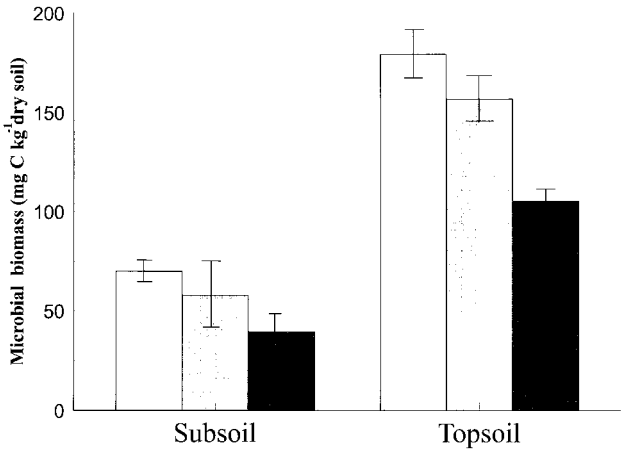

Figure 2. Microbial biomass of Deep Slade topsoil (soil B) and subsoil (soil A) measured (□) initially, and after incubation for 24 weeks with (■) ethoprophos or (▒) carbofuran.

3.2 Degradation of carbofuran in soils from the UK with carbofuran pretreatment histories

The degradation patterns of carbofuran in previously treated and untreated soils from the UK are shown in Fig 3. Residue data from all replicate samples (when available) are shown individually to provide an indication of sample-to-sample variability. The degradation rate of carbofuran was very fast in those soils last treated in the field one, two or four years previously in which no carbofuran residues were detected 13, 15 and 18 days after the initial application, respectively. In all three examples, degradation occurred more slowly in the respective control sample. In contrast, no difference in dissipation rate between pretreated and control soils was observed when the last carbofuran application in the field had been six, eight or 10 years previously. It appears that the rapid degrading ability induced by a single application of a granular formulation of carbofuran, applied at the commercially recommended dose, can persist for at least four years. Previous studies have shown that a normal application of carbofuran in the field can induce accelerated biodegradation to a level sufficient to reduce the biological performance of a new carbofuran application five years later.[22] Cabbage root fly and carrot fly can be active in the field for several weeks, hence adequate persistence of carbofuran is essential for effective control.[22] However, complete degradation of carbofuran was evident in our studies in two to three weeks in soils last treated one, two or four years previously. Degradation in control soils was also complete within four to five weeks in some circumstances (Fig 3), which suggests that, even in the absence of significant enhancement, the persistence of carbofuran in soil may be marginal to the requirements for effective pest control. Field studies have indicated a loss of carbofuran activity in one to two weeks following application to an 'enhanced' site in the field, whereas activity in a 'non-enhanced' soil was evident for five to six weeks.[23]

3.3 Carbofuran degradation in soils from Greece with carbofuran treatment history

The dissipation patterns of carbofuran in soils from Greece with known carbofuran pre-treatment histories are illustrated in Fig 4. Carbofuran degraded faster in soils which had been treated once only, six and 18 months ago, and in the soil treated annually for the last eight years, than in the corresponding previously untreated sample. In contrast, there was no difference in degradation rate between controls and soils treated once only, three years before. It appears that the effect of carbofuran applications in the field at the recommended dose can affect the degradation rate of a subsequent carbofuran dose for at

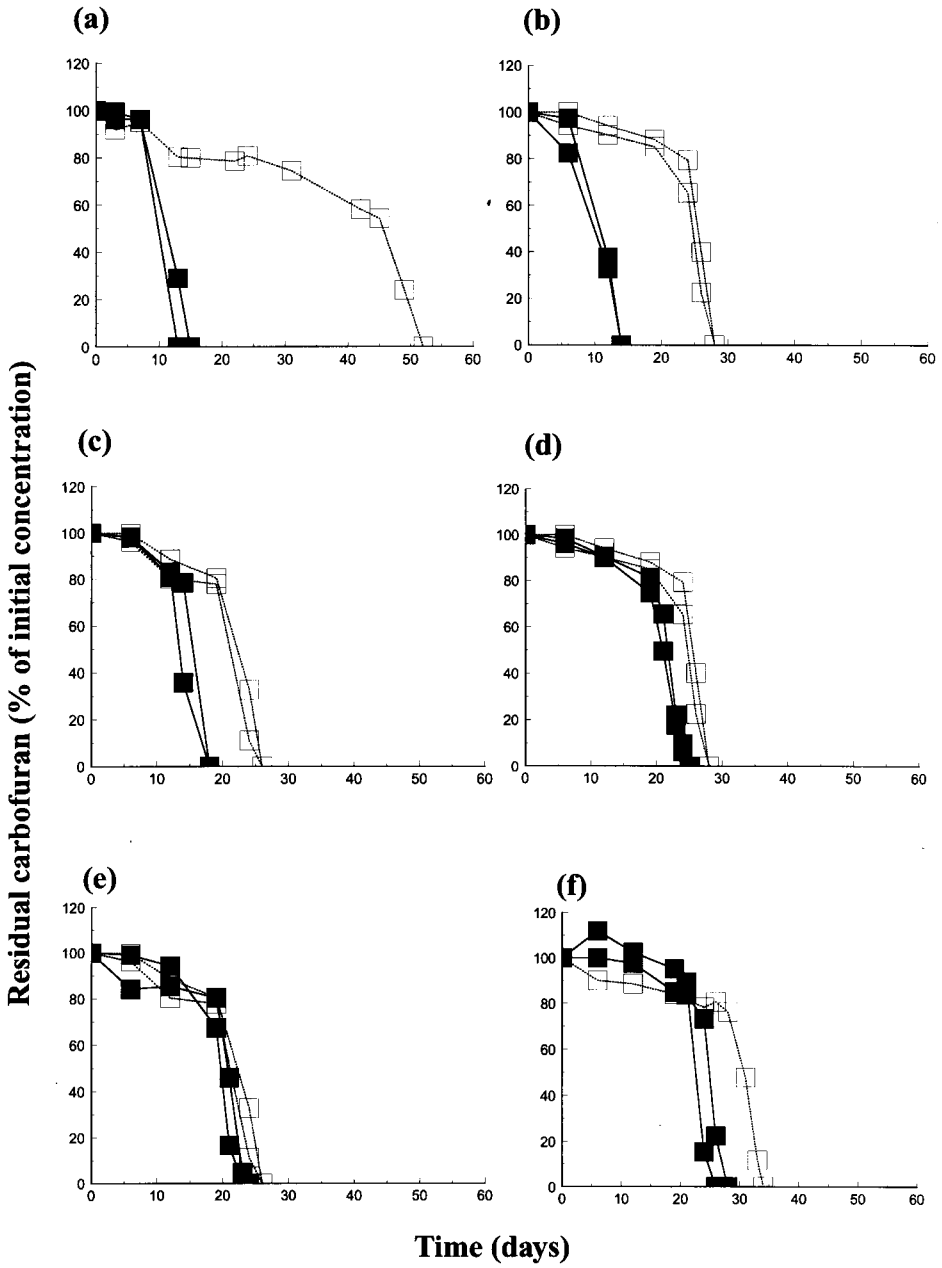

Figure 3. Degradation of a fresh carbofuran addition in soils from UK last treated with (■) carbofuran (a) one, (b) two (c) four (d) six (e) eight and (f) 10 years ago and (□) in control soils.

least 18 months but not for a period of three years. This contrasts with the results from the UK soils discussed above. Possible explanations for this are the extremely dry, hot summer soil conditions in Greece which do not favour the growth of soil bacteria, and the different crop management systems in soils from Greece, where maize or maize/cereal rotations are the main crop practices. Another major difference between the soils from Greece (Fig 4) and those from the UK (Fig 3) is the high pH of the former (Table 1). Under alkaline conditions, chemical hydrolysis may contribute to carbofuran dissipation.[11] However, the data in Fig 4 strongly suggest that biodegradation was the dominant mechanism, since degradation was characterised by an initial lag period followed by rapid rates of loss which would not occur if abiotic factors were responsible. Also, the overall loss patterns in the control soils (Fig 4) were very similar to those observed in several of the control soils from the UK (Fig 3), even though the pH of the latter soils was about two units lower, providing little likelihood of hydrolytic activity. The contribution of abiotic degradation could be examined by comparing rates of loss in sterile and non-sterile soils,[11] but this was not done in the present experiments. The present results are restricted to just a few field sites, and clearly far more data are required before firm conclusions concerning possible geographical differences in behaviour can be reached.

The data in Fig 4 provide the first evidence of enhanced degradation of carbofuran in soils from

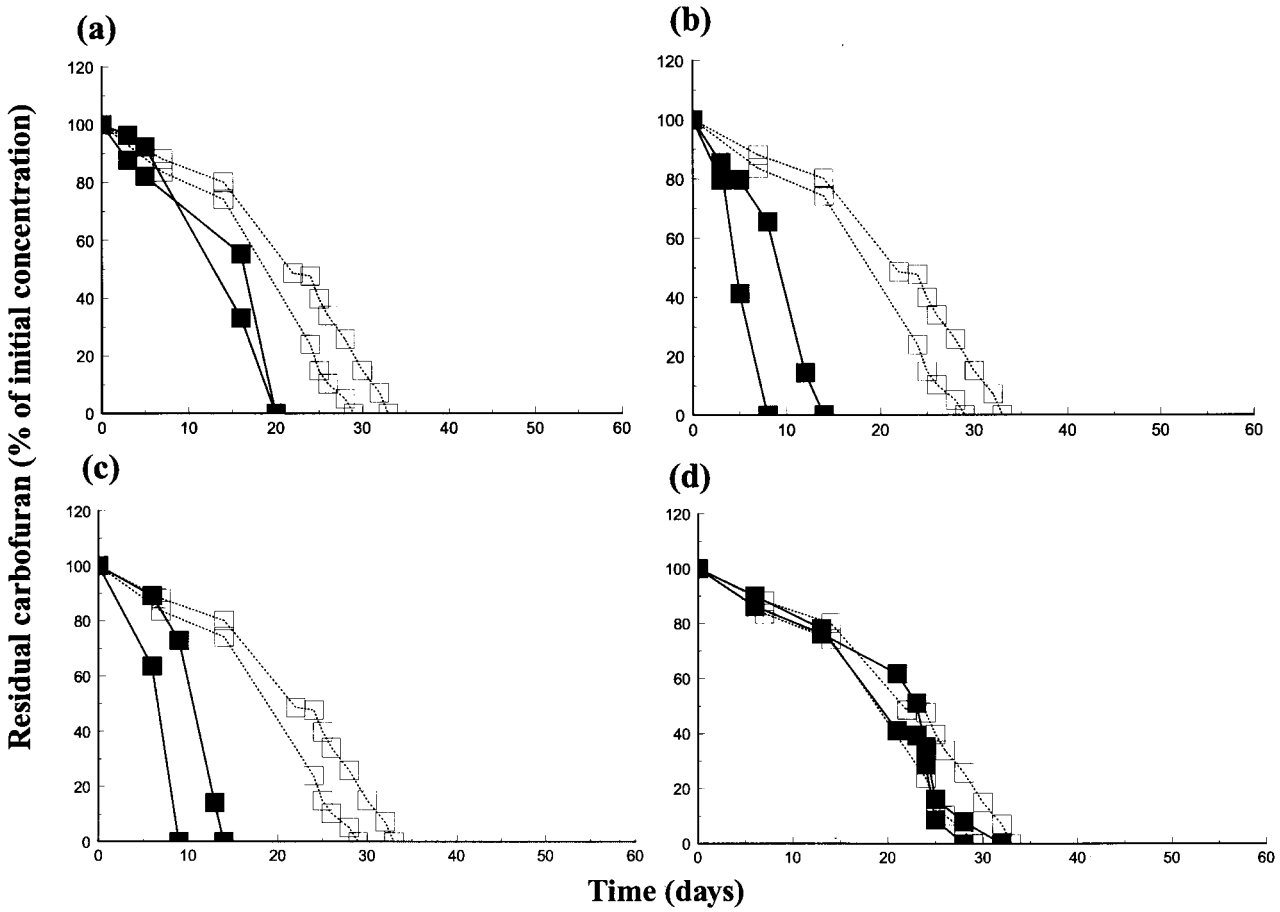

Figure 4. Degradation of carbofuran (■) in soils from Greece with different carbofuran treatment histories and (□) in control soils. (a) Annual carbofuran application for the last eight years; (b) last carbofuran application six months ago; (c) last carbofuran application 18 months ago; (d) last carbofuran application three years ago.

Greece, and there have been no reports of reduced biological activity of the insecticide under practical field conditions. Previous findings suggest that toxic levels of carbofuran must remain in the soil for approximately four to eight weeks after its application in order to ensure successful control of corn rootworm.[24] However, in soils from Greece last treated with carbofuran six and 18 months ago, carbofuran persisted for less than two weeks under incubation conditions similar to the average soil temperatures and moisture contents that are likely in the field. In the controls, degradation was complete in about five weeks. The main factor which may contribute to the lack of problems of diminished performance as a result of enhanced degradation in the field is the standard crop management practice in Greece of a one-year rotation of corn/cereals with use of carbofuran every two years. This time interval may well be adequate to prevent enhanced biodegradation problems (Fig 3), once more providing a possible contrast with the situation in the UK.

3.4 Ethoprophos degradation in soil from Greece with previous ethoprophos treatment

The degradation patterns of ethoprophos in the soils from Greece with a history or no history of ethoprophos application are shown in Fig 5. Data from the two replicates are again presented to illustrate the low variability in the data. There was rapid degradation of ethoprophos in the previously treated soils with 50% loss within about four days. In contrast, in the untreated soil, a long lag phase of about 35 days was followed by rapid degradation, and the time for 50% loss of the initial amount was >35 days. This is the first report of enhanced degradation of ethoprophos in soils from Greece, although enhanced degradation of ethoprophos has been reported previously in soils used for potato cultivation in the Netherlands where the fields had been treated with

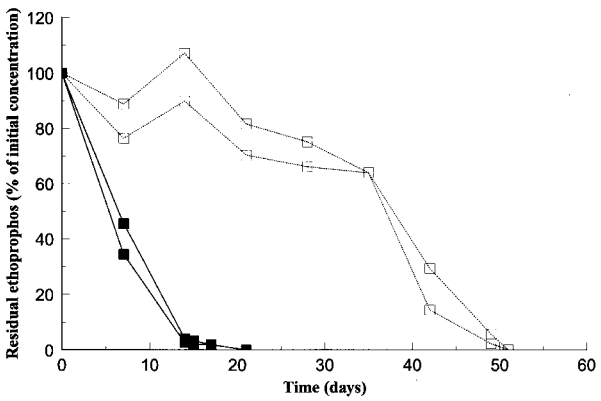

Figure 5. Degradation of ethoprophos in soils (■) with history and (□) no history of ethoprophos treatment.

the nematicide either annually, or once every two years, for the last four years.[12] According to our results, a single ethoprophos application within the preceding 12 months may be sufficient to activate the soil microflora for degradation of ethoprophos in the field. These results are in marked contrast to the laboratory studies with the UK top soil, where no enhancement of biodegradation was induced. The reasons for this difference in behaviour are not apparent at the present time.

3.5 Ethoprophos and carbofuran degradation in Greek soils from potato monocultures

The degradation patterns of ethoprophos and carbofuran in soil samples from Greece with known histories of ethoprophos or combined ethoprophos plus carbofuran treatment are shown in Fig 6, together with data for degradation in previously untreated samples from the same sites. Degradation of ethoprophos (Fig 6(a)) was very rapid in samples taken from a field which had received an annual application of ethoprophos for the last 30 years. This rapid dissipation of ethoprophos in these soils, less than 10% of the fresh ethoprophos addition was recovered seven days after treatment, is consistent with farmer complaints about reduced control of nematodes. The degradation rate of ethoprophos was much slower in the control soil and, unexpectedly, in the samples that had previously received only ethoprophos or a combined application of ethoprophos + carbofuran every year for the last seven years. However, soil analysis revealed a very low pH (4.4–4.5) in these samples, and previous studies with ethoprophos have suggested that enhanced degradation does not occur under acidic soil conditions, even with 10 annual applications of the nematicide.[25]

The degradation of carbofuran (Fig 6(b)) was very rapid only in the soil which had received repeated applications of ethoprophos. In this soil, carbofuran residues had declined to almost 10% of the initial amounts within 16 days, and had degraded completely within 19 days. In the control soils, a period of 80 days was required for complete degradation of the insecticide. This unexpectedly fast degradation of carbofuran in soils with no record of carbofuran application may be explained by the annual use of the carbamothioate herbicide EPTC (Eptam 10G) in this field over the preceding 30-year period for the control of annual grass weeds. Previous studies have suggested no cross-enhancement of degradation between EPTC and carbofuran,[7] but others have shown that this can occur in soils treated with other dithiocarbamates like triallate, suggesting that the situation with EPTC can be analogous.[26] The dissipation rate of carbofuran was very slow in the other soils used in this study (Fig 6(b)). In these samples only 30% of the carbofuran was degraded after incubation for 80 days, even though carbofuran had been applied regularly in the field previously. As discussed above, this almost certainly links with the extremely low pH of these soils (4.4–4.5) which is not favourable for bacterial activity. Enhanced degradation of carbofuran[2,27] or organophosphorus insecticides[25] and dicarboxamide fungicides[28] has also been shown to be very restricted in soils with low pH (<5.4).

The effect of antibiotics and soil fumigation on the degradation rate of ethoprophos in samples of one of the soils with the ability to degrade ethoprophos is shown in Fig 7. These results indicate a rapid loss of ethoprophos in the untreated samples, confirming the results in Fig 6(a). They also show rapid loss when treated with the antifungal antibiotic chloramphenicol. More limited degradation occurred when the soil had been fumigated or treated with the antibacterial antibiotic chloramphenicol. The above results confirm that soil micro-organisms are involved in the rapid degradation of ethoprophos in

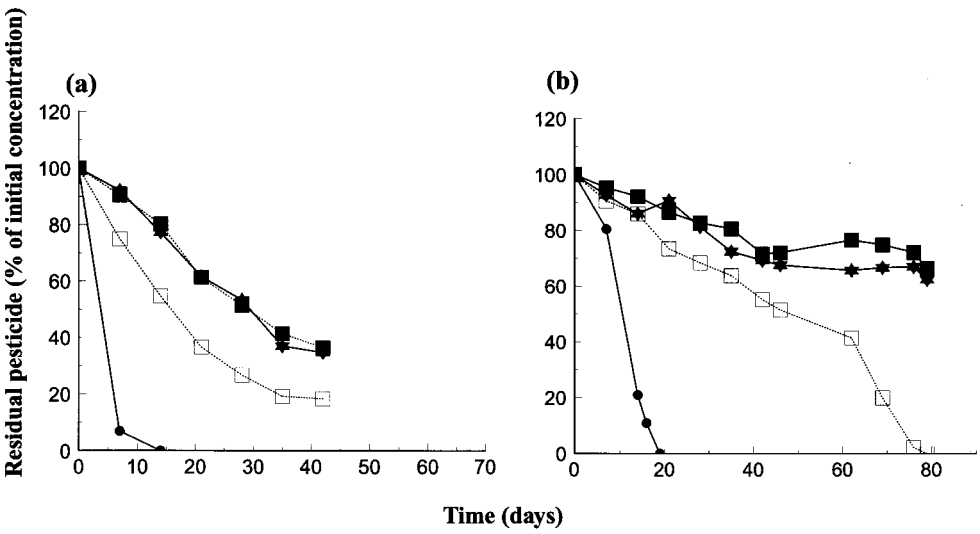

Figure 6. Degradation of (a) ethoprophos and (b) carbofuran in soils from potato cultivation in Greece with history of annual ethoprophos application (●) for the last 30 years, (■) for the last seven years, (★) with history of combined ethoprophos – carbofuran application for the last seven years and (□) untreated control soils. The results at each sampling time are the mean of two replicates.

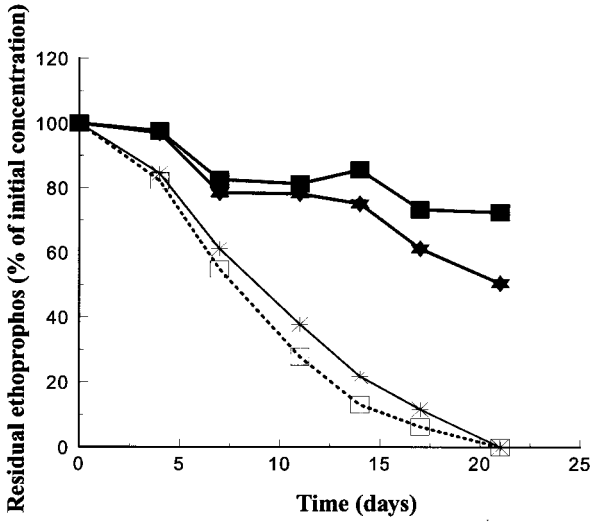

Figure 7. Degradation of ethoprophos in an 'enhanced' soil treated with (★) chloramphenicol, (∗) cycloheximide, (■) fumigated with chloroform, or (□) untreated.

pretreated soils and strongly suggest the involvement of bacteria rather than soil fungi. In previous studies of the microbial degradation of organophosphates, an *Arthrobacter* sp which was able to use isofenphos as sole carbon source was isolated from an enhanced soil,[29] and a microbial consortium consisting of at least six bacteria was reported to rapidly degrade fenamiphos.[30]

4 CONCLUSIONS

These results indicate complex interactions between soil chemical and microbiological properties, pesticide pre-treatment histories, cropping practices, and possibly geographical location, in determining the expression and significance of accelerated biodegradation of different soil-applied pesticides.

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